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OBITUARY.

THE LATE THOMAS CHARLES NOCK.

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WE have to record with deep regret the death of Mr. T. C. Nock which occured on 27th October 1923 at the Radium Institute London.

Mr. Nock was born in Ceylon on September 1st, 1883, and was the son of Mr. W. Nock, formerly Curator of the Hakdala Gardens, Ceylon.

Mr. Nock was employed as a Surveyor by the Government of Ceylon from 1901—1903, and came to the Straits Settlements in 1904 in the same capacity. He was appointed Assistant Inspector of Coconut Trees in Selangor in 1907, and was transferred to the Federal Government in 1908. In 1915 he was appointed Acting Assistant Agricultural Inspector, and in 1921, was confirmed in the appointment.

During his tenure of Office Mr. Nock earned the regard of all who came into contact with him by his unfailing courtesy, cheerfulness, and willingness to assist specialists with his extensive knowledge of general agriculture in Malaya.

The Officers of the Department offer their deepest sympathies to the family of Mr. Nock in their bereavement.

STUDIES ON HEVEA LATEX.

I. COAGULATION.

By W. N. C. Belgrave.

THE practical importance of latex coagulation led to much work in the experimental years of factory technique from 1911 to 1915; since methods have become fixed, less attention has been given to the subject. The necessity of a clear understanding of the process remains however as important as ever, and in addition some new cognate problems have arisen; of these the preservation of latex, and the problem of brown bast may be cited.

General.

Latex as it issues from the tree is well known to consist of an enormous number of hydrocarbon globules, for the most part spherical, of sizes varying from 2—0.5 microns (1 micron = .001 mm), in vigorous Brownian motion, suspended in an aqueous medium which is a dilute solution of nitrogenous (protein) bodies, sugars l-methyl inosite and mineral salts. There are also present bodies of a resinous nature, one or more alcohols akin to phytosterol, and a small amount of a lecithin-like substance.

"Coagulation" is generally taken to mean the separation and coalescence of the rubber globules to a coherent, somewhat elastic clot leaving, when the process is complete, a perfectly clear liquid—serum. Beadle and Stevens[1] and Whitby[2] pointed out that separation could take place without the formation of a clot, and that either 'creaming,' 'flocculation,' or 'coagulation' could occur depending on the size of the aggregates of globules formed on separation, the numbers composing the aggregates increasing progressively up the series.

In the tropics, coagulation (in the strict sense) usually commences within a few hours of outflow and progresses steadily from 12—24 hours. At the same time there is considerable bacterial activity resulting in the development of acidity and evolution of some CO_2. The perfection of natural coagulation varies according to the conditions, whether anaerobic or aerobic—in the former it is usually perfect or nearly so, in the latter secondary putrefactive changes occur, resulting in the production of alkalinity and partial coagulation only. Artificial coagulation may be brought about in varying degrees of perfection by small quantities of dilute acid, neutral salts of the di- and trivalent metals, heat, an electric current, and protein precipitants. Coagulation is inhibited or retarded by some antiseptics, a low temperature and alkalies.

The object of this paper is to put forward a restatement of the position, together with a simple physico-chemical theory of coagulation which, it is believed, fits the observed facts at least as well as the

only other consistently stated theory—that of enzyme action. Other writers who have rejected the enzyme theory, have not clearly stated how certain well defined phenomena can be explained.

It is proposed to consider first the state of rubber in latex, then the current theories of coagulation, followed by the writer's theory, and some practical deductions to be drawn therefrom. For the purpose of reference full use will be made of Whitby's comprehensive work—"Plantation Rubber" which gives an admirable account of the position in 1920.

The State of Rubber in Latex.

There has been considerable discussion on the physical state of rubber in latex, that is, whether it is a solid or liquid. As Whitby points out opinion now tends to the former view. De Vries[3] has recently stated that the matter has ceased to be of importance with the changing views of heterogenous systems, the old emulsion suspension anthithesis having given place to that of lyophile—lyophobe disperse phases, with caoutchouc in the former class due to a coating of protein. From one point of view this is correct, but from another it is of some importance to know whether separation of the rubber substance is due to physical causes only or whether in addition there are chemical changes such as polymerisation.

De Vries[4] has himself supplied an indirect proof of the absence of fundamental change by calculating the specific gravity of the rubber substance in latex from the specific gravities of latex and serum and comparing it with that of coagulated rubber. The writer has developed two other lines of work; one the volume changes, the other the temperature changes on coagulation.

1. *Volume Changes on Coagulation.*

A test tube of 250ccs capacity, fitted with a cork and narrow bore tube was filled with latex (D.R.C.* 20%) to which 2% of 10% acetic acid had just been added. After coagulation, which was rapid and complete, water was added from a burette to bring the volume back to a mark on the narrow tube.

Two experiments gave contractions of:—(a) 0.5ccs, (b) 0.3ccs, and these are well within the limits of experimental error due to air bubbles included on filling the tube and liberated on coagulation.

Taking a mean figure of 0.4ccs as the contraction, and (for this purpose) the specific gravity of rubber before and after coagulation as 1.0, we have

$$\text{Volume of rubber} = \frac{20}{100} \times 250 = 50 \text{ ccs.}$$

i.e. the change of volume on coagulation does not exceed 0.8%, a negligible figure.

* D.R.C. = Dry rubber content.

2. *Temperature Changes.*

A cylindrical Dewar vacuum flask was used as calorimeter, with a thermometer graduated in 0.1°C and reading by estimation to 0.01°C. The water equivalent of the calorimeter when half-filled was 21 grms and the rate of change over the range 20—30°C (air temperature—26°C) did not exceed 0.02°C per minute.

In order to ensure rapid coagulation and consequent minimum heat loss, 10% of strong hydrochloric acid was used for coagulation.

Preliminary experiments showed that the heat evolved on dilution of the strong acid caused a rapid rise in temperature, followed by a slight fall as the calorimeter came into equilibrium, and then a slow steady rate of cooling, which was attained in less than 2 minutes.

On the addition of HCl to latex there was a similar rapid rise and initial fall, but after 2—3 minutes as the latex could be felt to thicken, a slight but constant rise was observed; at the end of the 5th minute coagulation had proceeded to such an extent as to immobilise the stirrer. No further rise took place, and it seems fair to take the extent of this second rise as the extent of the temperature change on coagulation.

Two experiments gave:

100 ccs of strong (40% D.R.G.) latex and HCl.

Interval.	(a) Temperature.	(b) Temperature.
0'	25.10°C	25.05°C
0.30"	28.50	28.30
1	27.50	27.40
2	27.40	27.75 thickens
3	27.50 thickens	27.85
5	27.95	27.80 coag.
10	27.90	27.75
	0.55°C	0.45°C

that is a mean second rise of 0.5°C for the coagulation of 40 grms of rubber, or assuming a specific heat for latex equal to that of water (too high a value) heat evolved = (110 × 21) × .5 = 65.5 calories or a maximum possible change of 1.6 calories per gram of hydrocarbon, which is such a small quantity as to suggest that no deep seated chemical change or change of state has occurred, that is that rubber in latex exists as a solid.

Thus both lines of work confirm the modern view that caoutchouc exists as such in latex, and that coagulation may be regarded as a problem of separation and coalescence uncomplicated by more radical changes.

Theories of Coagulation.

(a) *Bacterial.*

The bacterial theory of coagulation, expounded by Eaton and Grantham[5] and Vernet[6] holds that natural coagulation is due to acid formed by the action of bacteria.

In support of this the following facts are adduced.

(*a*) Latex[5] was heated in plugged flasks in an autoclave for brief periods (brief to avoid heat coagulation). Flasks kept plugged remained fluid, which those afterwards exposed to infection coagulated. It has been objected that the period of heating was insufficient for certainty either of sterilisation or enzyme destruction, but the objection seems invalid if the experimental results are accepted, since it would be a remarkable coincidence that flasks left unplugged were insufficiently while plugged flasks were sufficiently heated.

(*b*) Vernet reports that latex collected by him in the field under sterile conditions remained sterile.

A considerable number of observations were made by the writer on this point. Bacteria where present, were demonstrated by making a cover-slip smear of latex or serum, drying, fixing by heat and staining with hot Carbol-Fuchsin, washing in water, drying, soaking in xylol (to swell and dissolve or render transparent the rubber film) and mounting in Canada Balsam.

All attempts at collecting sterile latex from the tapping cut failed, and eventually the following procedure was adopted — the outer scaly dead bark was removed and the tree surface scraped till latex just started to exude in minute drops; the exposed part was then scrubbed with 96% alcohol, and the last traces of alcohol flamed off, while still burning a cover was applied—(a Graham dialyser was found convenient), then a sterilised (alcohol-flamed) gouge was used to remove a cylinder of bark about $\frac{1}{2}$" diameter. The out-flowing latex was collected in a sterilised pipette and placed in a small sterilised test tube, the usual precautions being observed.

Even with such precautions, a considerable percentage of failures is to be expected because of the many opportunities for infection. The following are typical of the results obtained :—

30- 9-21.	Four tubes put up.
1-10-21.	One coagulated — bacteria present.
	3 fluid — one examined, bacteria absent.
3-10-21.	2 remainder coagulated — bacteria present.
3-10-21.	3 tubes put up.
5-10-21.	2 coagulated — bacteria and gas.
	1 fluid—no bacteria.
11-10-21.	4 tubes put up.
12-10-21.	All 4 tubes coagulated — all bacteria.
17-10-21.	6 tubes put up.
20-10-21.	2 remained fluid no bacteria, bacteria in those coagulated.

A total of 50 tubes was examined, in approximately 30% of cases there was delayed coagulation associated with delay in appearance of bacteria.

Such results are evidence that bacteria play an important part in coagulation of latex, but cannot be considered absolutely to exclude

the operation of an enzyme in the coagulation of latex obtained in the usual way, since a higher proportion of sap from the cortical tissues must be present in the latex used in these experiments and this might inhibit enzyme action.

(*c*) Another observation in favour of the bacterial theory is the well-known early coagulation of latex on wet mornings. The writer has in such cases invariably found the coagulated latex to be swarming with bacteria ; due doubtless to a high density of infection derived from water running down the bark and over the tapping cut.

The three lines of evidence given above are strongly in favour of a bacterial theory of coagulation, but such a theory in its simplest form fails to provide a complete explanation of observations by Whitby, Barrowcliff and Campbell.

The Enzyme theory.

Whitby[2] considers that the action of small quantities of dilute acids — whether added as such, or formed by bacterial action — in bringing about coagulation is indirect and is due to the activation of an enzyme. In support of this, the following observations are adduced :—

(1) Latex coagulates readily in the presence of chloroform or toluene, antiseptics which are considered not to inhibit enzyme action, but does not coagulate in presence of formalin, which is known to paralyse or destroy enzymes, in addition to acting as an antiseptic. Cyanides also inhibit coagulation.

(2) Campbell[7] has shown that calcium salts accelerate coagulation to a much greater extent than barium salts. This is correlated with the known acceleration of the clotting of milk and blood by calcium salts. Conversely the calcium precipitants, oxalates or fluorides, markedly inhibit coagulation.

(3) Barrowcliff[8] has shown that latex may be dropped into boiling water without the occurrence of coagulation ; on standing or on the addition of small quantities of acid, coagulation does not occur, but may be brought about by the addition of a drop of fresh untreated latex.

(4) Whitby[2] has demonstrated the existence of a 'gap' in acid coagulation, small quantities of acid bringing about coagulation, while larger quantities of the stronger acids inhibit it, still larger quantities again cause coagulation which is now very rapid. This is assumed to be due successively to enzyme acceleration, enzyme inhibition, then to physical action.

(5) The slowness of action of small quantities of acid is taken by Whitby[2] as evidence of enzyme action.

(6) In general, a strong analogy to the clotting of milk and blood is claimed.

It is proposed to examine these claims in detail.

(1) *The action of antiseptics.*

The assumption here involved has been found to be incorrect, neither toluene, chloroform nor thymol functions as an antiseptic in presence of latex.

20ccs of concentrated (32% D. R. C.) and dilute (16.5% D.R.C.) latex was put up in open test tubes with :—

(*a*) 1cc chloroform (*b*) 1cc toluene (*c*) .5 grs. powdered thymol, (*d*) (*e*) and (*f*) with 5 ccs of saturated aqueous solutions of the above. The (*a*) (*b*) and (*c*) tubes showed small clots after 1 hour, the remainder were normal—next day all the tubes showed coagulation and abundant bacteria were present.

Similar tubes with the addition of .5 ccs formalin remained fluid and sterile.

In addition to antiseptic action, formalin is known to have a specific action on proteins, retarding their coagulation. Some such action on latex is suggested by the fact that more acid i.e. a higher hydrogen ion concentration is required to bring about coagulation in formalin-preserved than in normal latex. The total quantity however, is still within the limits which are said by Whitby to act indirectly only — yet the enzyme is supposed to have been inhibited.

In the case of cyanide, addition of acid sufficient to overcome the buffer action of the weak acid results in coagulation at the usual hydrogen ion concentration i.e. cyanides have no specific action.

TABLE I.

An approximately 2% solution of commercial potassium cyanide was neutralised to phenol red with HCl.

10ccs of the solution diluted to 50ccs with water, required 2.5cc N/10 HCl to bring it to pH 4.6 (methyl red). 10ccs of solution was then added to 50ccs of dilute latex (D.R.C. = 20%) and N/10 HCl added.

	50ccs latex.	50ccs latex + 10ccs neutral cyanide.
+ 3ccs N/10 HCl.	5.3 pH	5.7 pH
+ 4	4.9	5.4
+ 5	coagulates	5.4
+ 6	,,	5.2 Thickens
+ 7		5.0 ,,
+ 8		coagulates

i.e. an excess of 3 ccs of acid is required for coagulation corresponding to the theoretical difference of 2.5ccs due to the added cyanide.

The obvious explanation of the failure of some of the antiseptics is absorption or possibly solution in the hydrocarbon globules, just as the disinfecting power of mercuric chloride may be materially reduced in presence of proteins.

(2) *Influence of salts.*

Campbell's observations on the preponderating influence of calcium over other neutral salts, while suggestive, is not proof of enzyme action. Recent work has shown that the "valency law" is only a first approximation, and that there are pronounced differences in equivalent precipitating power among metals of the same valency, *e.g.* Lewis (p. 34) quotes the work of Oden on the precipitation of colloidal sulphur, which showed that the precipitating power of caesium chloride was approximately one hundred times that of lithium chloride, and that of barium chloride was equal to that of strontium chloride and double that of calcium chloride. The differences found are ascribed to different degrees of adsorption, and except by analogy with milk and blood there seems to be no valid reason for assuming the specific action of calcium salts to be due to activation of enzyme action and not to selective adsorption; there are however, further facts to be considered.

Campbell found using 1% solutions of calcium and barium chlorides, that 1—5cc. Ca Cl_2 to 1000 of latex (30—35% D. R. C.) produced no effect in 3 hours, while with 10cc small clots formed in 20 minutes, with 25cc coagulation started in 10 minutes, and 50ccs caused coagulation to start immediately. 1—25cc of Ba Cl_2 produced no coagulation in 3 hours, and 50 cc caused coagulation to start in 10 minutes.

The form of calcium chloride used is unfortunately not particularised hence it is impossible with certainty to recalculate these results on the only true basis, viz. effect of equivalent (gram-molecular) solutions. If anhydrous Ca Cl_2 was employed, in equal volumes calcium was present in more than double the equivalent concentration of barium (Ca Cl_2=110; Ba $Cl_2 2H_2O$=243); on the other hand if $CaCl_2 6H_2O$ was employed the equivalent concentrations were nearly equal (Ca Cl_2 $6H_2O$=218.) In the former case, Campbell's results would need no further discussion.

The writer found however, that when molar solutions were used, that for limiting quantities calcium chloride was more efficient than barium chloride—the figures approximating to those of Campbell. There are two further considerations:—

(*a*) If the effect of the metallic ions is not additive, but is specific, whether acting physically or through an enzyme, the calcium normally present in latex would assist added calcium, but not barium.

Campbell found that 1% of 1% calcium chloride just started coagulation—assuming that he used the hydrated salt, this would give an addition of .01 x 56/218, grams of Ca expressed as Ca 0=.0026 grs of Ca 0. and this is only a 25% increase on the *dialysable* calcium found by Beadle and Stevens* (Ca 0=013%).

(*b*) The smaller solubility of barium salts compared with those of calcium must be considered. The same authors showed that the *dialysable* sulphate in latex (as SO_3) was .008%. Calcium sulphate

* Whitby *Plantation Rubber* p. 62.

is soluble to the extent of 0.2% in water and so will not be removed in the concentrations used in these experiments, but barium sulphate is in a different category ; 0,008 grs of SO_3 will precipitate the barium in $\frac{243}{80}$ x .008 grs=.025 grs. and this is contained in 2.5ccs of a 1% solution i.e. 25 ccs of such solution must be added to 1000 ccs of latex before barium ions will be present in the solution to any appreciable extent—this alone amply suffices to explain the greater apparent efficiency of calcium, without taking into account the possible formation of organic salts.

If it is admitted that calcium salts may physically assist in coagulation, the action of calcium precipitants would follow. There is however the possibility of physical action ; just as salts of divalent metals accelerate, so salts of divalent acids may inhibit coagulation. Unfortunately for work on the particular point, practically all di- and tri-valent calcium salts are insoluble in water ; there is however one exception,—calcium sulphate is soluble to the extent of 0.2% at 30°C and as the percentage of dialysable calcium as oxide in latex is in the neighbourhood of 0.02%, any action of sulphates cannot be due to precipitation of calcium. To make certain that the excess of added sulphate does not cause precipitation, the following experiment was tried.

4ccs of $\frac{M}{10}$ $CaCl_2$ solution (=.022 grs Ca O) was added to 100 cc water and then 2cc 2M Na_2SO_4 was run in, no precipitate resulted. (On addition of 2cc M potassium oxalate, calcium oxalate was precipitated). The effect of sulphate was then tested as in Table II.

TABLE II.

Half-strength latex (D.R.C. = 20%) was used in each experiment.

		State after		
	pH value.	5'	2 hr.	24 hrs.
50cc Latex + 3ccsN/10 HCl	5.0	Thick.	Coagulated.	—
„ „ + acid + 0.2ccM. Na_2SO_4	5.0	„	„	—
„ „ + „ + 0.4 „ „ „	5.0	Fluid.	Half-coag :	coagulated.
„ „ + „ + 1.0 „ „ „	5.1	„	Fluid.	do
„ „ + „ + 2.0 „ „ „	5.1	„	„	do
„ „ + „ + 4.0 „ „ „	5.4	Thick.	Coagulated.	—

After 24 hours all coagula showed abundant bacteria.

TABLE III.

Action of oxalates.

Latex as in Table II. Molar $K_2C_2O_4$ neutralised to phenol red by addition of HCl.

	pH after addition.	State after 5'	State after 1 hr.	24 Hrs.	pH after 24 hours.
50cc + 3cc N/10 HCl	5.0	Thick	Coagulated	—	—
50cc + acid +1cc oxalate	5.4	Slight thickening	Half-coagulated	Coagulated	5.2
50cc + acid +3cc oxalate	5.6	Fluid	Fluid	Coagulated milky serum	5.1
50cc + acid +5cc oxalate	5.8	„	„	Half-coag.	5.3
50cc + acid + 10cc oxalate	6.0	„	„	„	5.3
Latex + 1cc oxalate	6.0	Fluid	Fluid	Coagulated	4.9
Latex + 3cc oxalate		„	„	Coagulated milky serum	5.3
Latex + 5cc oxalate		„	„	Soft coagulum very milky serum	5.3
Latex + 10cc oxalate		„	„	Half coagulated	5.4

Bacteria were abundant.

It will be seen from the above Tables that both sulphates and oxalates clearly have an inhibiting action on the immediate thickening of latex by small quantities of acid, while only oxalates inhibited coagulation after 24 hours, corresponding to this sulphates have no buffer action, while oxalates have a marked action in the range dealt with.

It is suggested that the fact that sulphates, which have been shown not to precipitate calcium in the concentration in which it is present in latex do inhibit coagulation materially weakens the evidence of oxalates in favour of enzyme action.

(3) *Barrowcliff's experiment.*

Barrowcliff, and Whitby who confirms his results, have overlooked an important fact in stating that latex dropped into boiling water, no longer coagulates in the ordinary way. It does not *coagulate*, but on standing, or on addition of the usual proportion of dilute acid, it clearly *flocculates*, and separation is so complete that filtration can readily be effected. The filtered portion with slight manipulation between the fingers forms a coherent clot with can be machined. Whitby admits

that creaming, floculation, and coagulation are merely different stages of aggregation, and it can scarcely be argued that the function of the hypothetical enzyme is merely to take separation from the stage of flocculation to that of coagulation. Further considerations are :—

(*a*) Much better aggregation can be obtained from Barrowcliff's fluid if the latex before dropping into boiling water is made slightly alkaline. (Table IV).

(*b*) Small quantites of calcium salts in presence of dilute acid bring about better coagulation than barium salts (Table V.)

(*c*) The writer has been unable to repeat Barrowcliff's result that very small quantities of fresh latex—"a few drops"—brings about normal coagulation, instead appreciable quantities have been necessary. (Table IV.)

TABLE IV.

Latex was dropped into boiling water, which was kept boiling, in the proportion of 20 latex : 25 water. After cooling, surface scum was removed. 'Alkali' latex had 10% of its original volume of Normal Na OH added, and after cooling an equal volume of N. HCl.

TABLE IV.

	Normal Latex.			Alkali Latex.
	After 1 hr.	After 20 hrs.	After 20 hrs.	After 20 hrs.
	Sample A.		Sample B.	Sample B.
100 ccs — Control	Fluid	Separation, no clot	Separation	Separation
100 ccs + .8ccs N. HCl	Thick	Separation, no clot	„	Separation, with slight clotting on stirring
100 ccs + 2.0 ccs N. HCl	...	...	Fair clot on stirring	Excellent clot
100 ccs + .5 ccs M. $CaCl_2$	Fluid	Separation, no clot	Separation, no clot	Half coagulated good clot
100 ccs + 1.0 ccs M. $CaCl_2$	Thick	Separation, no clot	Separation, no clot	„
100 ccs + 3 ccs M. $CaCl_2$	„	Separation, loose clot	Separation, loose clot	Nearly complete, good clot
100 ccs + 1 drop fresh latex	Fluid	Separation, no clot	Separation, no clot	Separation, no clot
100 ccs + .2 ccs fresh latex	„	Separation, no clot	Separation, no clot	„ „
100 ccs + 4 ccs fresh latex	„	...	Half coagulated	Completely coagulated
100 ccs + 8 ccs fresh latex	„	Separation, no clot	...	.....
100 ccs + 12 ccs fresh latex	„	Soft clot	Half coagulated	Completely coagulated
100 ccs + 20 ccs fresh latex	„	Fair clot	Half coagulated	Completely coagulated

TABLE V.

Comparison of action of Ca Cl_2 and Ba Cl_2 on boiled latex.

		After 20 hours.
100ccs + .2ccs M. Ca Cl_2	...	Separation, no clot.
„ + .4cc „	...	„ „
„ + 1cc „	...	„ „
„ + .2cc M. Ba Cl_2	...	„ „
„ + .4 „ „	...	„ „
„ + 1.0 „ „	...	„ „
„ + .8 „ N. H. Cl		
„ + .8 „ „ + .2cc Ca Cl_2	...	Good Clot.
„ + .8 „ „ + .4 „ „	...	„
„ + .8 „ „ + 1.0 „ „	...	Perfect clot.
„ + .8 „ „ + 2.0 „ „	...	„
„ + .8 „ „ + .2 „ Ba Cl_2	...	Very loose clot.
„ + .8 „ „ + .4 „ „	...	„
„ + .8 „ „ + 1.0 „ „	...	Stringy clot.
„ + .8 „ „ + 2.0 „ „	...	Perfect clot.

4. *The gap in acid coagulation.*

This most interesting phenomenon is very strongly suggestive of the existence of an iso-electric point, to be discussed later, while the enzyme hypothesis seems to be put out of court by the existence of an exactly similar gap in latex treated by Barrowcliff's method. Latex was dropped into boiling water as in (3) above.

50ccs + 5cc N/10 HCl—rapid separation no clot.

„ + 3cc N.HCl—remained perfectly fluid, and so remained for 24 hours.

„ + 3cc Strong HCl—Immediate coagulation to good clot.

5. *Slow rate of normal coagulation.*

It is somewhat difficult to follow Whitby's line of argument in view of the very large number of chemical reactions and physical changes taking place at low velocity at ordinary temperature, and accelerated by changing concentration of one of the reacting substances. (a) The acceleration of rate of coagulation by increase of acidity is a perfectly smooth and regular event, (for the low side of the gap), and viscosity determinations show clearly that immediate changes, indicative of incipient coagulation, occur on addition of even very small quantities of acid. Again, after a few hours bacterial action will materially have increased the acidity of the medium, and the added acid can no longer be taken as a measure of acidity.

Table VI shows the viscosity changes referred to :—

TABLE VI.

As De Vries[3] has recently pointed out, the usual Ostwald viscosimeter is useless for latex determinations owing to traces of coagulation occurring. A simple pipette form of instrument was employed, which

could easily be cleaned. Determination were made at air temperature (26-29° C) and for convenience latex diluted with an equal volume of water (D. R. G.-18-20%) was employed. The specific gravity of such latex may for the purpose of this experiment be taken=1. Determinations with water were made at frequent intervals as a check. In all cases determinations were made as rapidly as possible after addition of acid. 50 ccs. was used in every case.

		9.6.23.	11.6.23.	13.6.23.	31.6.23.	20.7.23.
Water		0'.31"	0.32	0'.31"	0.31	0.30
Latex untreated		1'.25"	1.27	1.21	1.18	1.16
50ccs Latex	+1cc N/10 HCl	1.30	1.29	1.22	1.23	1.17
,,	+2 ,,	1.37	1.31	1.28	1.24	1.35
,,	+3 ,,	1.53	1.40	1.43	2.2	—
,,	+4 ,,	3.00 coag:	coag:	coag:	coag:	1.59
,,	+5 ,,	—				2.31 coag:
50cc Latex	+1cc N. HCl		lumpy.			
,,	+2 ,,	1.05	1.04			
,,	+3 ,,	1.05	1.05			
,,	+4 ,,	1.05	1.04			
,,	+5 ,,	1.08	1.04			
,,	+6 ,,	1.12	1.04			
,,	+7 ,,	1.25	1.05			
,,	+8 ,,	1.31	1.10			
50cc Latex	+1cc N/10 NaOH.				1.00	1.06
,,	+2 ,, ,,				0.59	0.59
,,	+3 ,, ,,				0.59	0.59
,,	+5 ,, ,,				0.56	0.57
,,	+1cc N				0.50	0.51
,,	+1.5 ,, ,,				0.48	0.55
,,	42.5 ,, ,,				0.50	0.57
,,	+3.5 ,, ,,				0.53	0.42
,,	+4.5 ,, ,,				0.49	0.58
,,	+5 5 ,, ,,					

All determinations were made between 9.00 and 10.00 a.m. At 1 p.m. all except the strong acid and alkaline latices were either coagulated or too thick for measurement. The exceptions gave practically unchanged values.

(5) *Analogy with clotting of milk and blood.*

The evidence brought forward tends decidely to weaken the analogy in question.

General.

In general it is claimed that none of the reasons hitherto brought forward in support of the enzymic theory of coagulation appear to be conclusive when subjected to critical examination; on the other hand a simple theory of bacterial action leading to the production of acid fails to explain all of the observed facts.

There is however, no doubt, that bacteria play some part, direct or indirect, in coagulation. It is possible that they function in one or more of three ways :—

(i) Specific substances may be excreted.

(ii) The protective colloid—protein—may be destroyed.

(iii) Acidity may be developed.

Possibility (i) is negatived by the fact that bacterial decomposition can be allowed to proceed far in latex without coagulation taking place if alkali is added from day to day (in amount sufficient to keep the pH value about 8.5). Judging by the foul smell which develops protein decomposition also is far advanced, but coagulation does not occur, till—either artificially by the addition of acid, or naturally by failure to add sufficient alkali—the acidity rises.*

If this experiment is taken to exclude (i) and (ii) we are left with (iii) viz :—the development of acidity as the sole mode of bacterial action, which leaves no essential difference between natural and artificial coagulation. Hereafter no distinction will be made.

General considerations on Emulsification.

It is known that some heterogeneous systems are extremedly sensitive to even minute quantities of salts while others are undisturbed by the presence of comparatively large quantities of salts of monovalent metals ; this formerly led to a distinction between highly sensitive *suspensions* in which the disperse phase was supposed to be solid, and less sensitive *emulsions* in which it was supposed to be liquid. This distinction no longer holds good, and the division is now between *lyophobe* systems in which the affinity of the disperse phase for the dispersion medium is low, and *lyophile* systems in which it is high.

Latex obviously belongs to the latter relatively insensitive class, the reason for this will be discussed later.

Recent work (summarised by Willows and Hatschek,[10] and Lewis[11]) has led to the view that for the continued existence of a heterogenous lyophile system such as latex the following conditions must be fulfilled —

(*a*) The particles of the disperse phase must be between certain limits of size.

(*b*) The particles constituting the disperse phase must be electrically charged.

(*c*) The surface tension of the dispersion medium must be below a certain critical value.

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* It may be of interest to to record that rubber so prepared gives a normal curve on the Schopper machine after vulcanisation, and has a low optimum time of cure.

(*d*) A protective layer must be present around the particles. Such a layer is formed whenever a substance capable of lowering the surface tension of the dispersion medium is dissolved therein.

The presence of such a layer,—almost invariably of a colloid —confers on the protected particles its own electrical properties. As a corollary we have the fact that when the protective body is amphoteric in nature *e.g.* is a protein, the existence of an *iso-electric point* is revealed. This is a point of hydrogen ion concentration at which the electrical charge on particles vanishes or becomes a minimum and at which there is maximum instability.

The electrical charges on particles on either side of the isoelectric point are found to be opposite in sign, being negative on the alkaline and positive on the acid side. From this it may be deduced that on the one side the metallic ions of neutral salts will exert a greater influence on separation, and on the other acid side that the anions will be of greater importance. This deduction is found in practice to be correct.

When the necessary conditions for stability are departed from beyond a limiting value, the stability of the system is destroyed, and separation—precipitation, clotting, clarification, coagulation—ensues.

General considerations applied to latex.

In the application of the general theory to latex one limiting consideration must be made clear—physico-chemical work is carried out with pure substances, under controlled conditions: latex is a biological fluid, subject to change, and containing not inconsiderable quantities of salts which *ex hypothese* have marked effects on the system under consideration. Deviation from rule must therefore be expected.

The phenomenon of coalescence of rubber globules as opposed to simple separation presents no theoretical difficulties in the case of a plastic solid (or viscous liquid) such as rubber. Once two particles collide, the interfacial tension will be exerted in the direction which tends to reduce surface energy to a minimum, and this is most easily effected by coalescence with the resultant reduction of surface; it is therefore proposed to consider only the necessary physical conditions for stability and separation.

All the conditions for stability have long been known to exist in fresh latex —the particles are electrically charged, their size is within the given limits, and proteins, among other substances, are present to lower the surface tension of the dispersion medium and to form a protective layer.*

Hydrogen ion concentration.

Since hydrogen ion concentration plays a most important part in the modern theory of heterogeneous mixtures, it is essential to follow as

* It is important to remember that this protective layer, due to absorption is *not* a definite lining membrane, such as Weber claimed to have demonstrated, but is merely an adsorption film.

completely as possible the changes undergone by latex in this respect. Total acidity is apt to be misleading, since owing to different degrees of dissociation, equivalent quantities of a 'weak' acid e.g. acetic and a 'strong' acid e.g. hydrocholoric may produce very different hydrogen ion concentrations in the same solution. For this reason little note was taken of total acidity and the 'strong' acid HCl and 'strong' base Na OH were used in the experiments given below. For convenience the pH notation has been adopted i,e. the negative logarithm of hydregen ion concentration per litre; on this notation large values mean low acidity, small values high acidity.

The determination of the pH value of latex is not easy, Electrometric methods are difficult and uncertain owing to surface coagulation on the platinised surface of hydrogen electrodes, while colorimetric methods must be very approximate owing to the opacity of the fluid, further, over the range of values from untreated latex to first coagulation point 6.0 to 4.8, no brilliant indicator colour was available. The method finally adopted, that of spotting. was found to give fair agreement with a few electrometric determinations, and results may be relied on to $\pm$.2. The indicators found most useful were thymol blue (both ranges) brom-phenol blue, methyl red, and phenol red. A comparison of the two methods is given in Table VII.

TABLE VII.

	Electrometric.		Colorimetric. (Me red).
	Strong. (36% D.R.C.)	Dilute. (18% D.R.C.)	Dilute. (18 % D.R.C.)
Latex untreated	6.2 pH	6.4 pH	6.0 pH
50cc." +.5cc N/10 HCl	6.2 ,,	5.95 ,,	6.0 ,,
,, + 1cc	5.75 ,,	5.8 ,,	5.7 ,,
,, + 2cc	5.55 ,,	5.4 ,,	5.5 ,,
,, + 3cc	5.35 ,,	5.1 ,,	5.2 ,,
,, + 4cc	5.2 ,,	4.9 ,,	5.1 ,,
,, + 5cc	5.1 ,,	4.8 ,,	5.0 ,,
,, + 6cc	—	4.75 ,,	4.9 ,,
		coag:	coag
,, + 9cc	4.9 ,,		
,, + 11cc	4.8 ,,		
	coag:		

A large number of determinations gave a pH range for fresh latex of 5.8 to 6.3; on standing in shallow layers, or on evacuation, this rapidly increased by .2 or .3 pH due to loss of CO_2.

Table VI showed clearly that changes were set up on the immediate addition of acid which resulted in increased viscosity, but for rapid coagulation (in 5-19 minutes) a pH value of 5. 2-4. 9 is necessary.

As the pH value is further decreased by the addition of further quantities of 'strong' acids e.g. HCl, HNO_3, H_2SO_4 coagulation becomes less perfect, and shortly the gap of Whitby is reached and equilibrium is regained; at the same time viscosity is markedly decreased. The quantity of HCl required for equilibrium is 4-6 ccs

N.HCl to 100ccs of dilute (20% D.R.C.) latex, and the pH value is about 2.5. With the addition of still more acid and at a pH beyond the lower limit of thymol blue, coagulation again occurs.

The electric charges on the particles.

V. Henri and others have shown by the method of cataphoresis, that the globules in latex are *negatively* charged, i.e. travel to the positive pole on the application of a potential difference. This has been confirmed by the writer both macro-and microscopically and the additional observation has been made that, within the gap, the particles are *positively* charged i.e. travel to the negative pole. The positive charge however always appears to be smaller that the negative, since the speed of movement of normal globules always exceeds those positively charged.

The action of salts.

A large number of experiments were carried out, which confirmed the results of other workers on ordinary latex, in addition latex within the gap was investigated.

(*a*) Ordinary latex pH. 5.8—6.2. The usual results were obtained; the kation plays the most important part in coagulation and Al in alum and Ca were the most efficient precipitants, followed by Ba, Sr, Co and Mg. K, and Na did not cause separation until added in large quantities, while the addition of .2-1ccs of M. solutions of salts of the metals of higher valencies to 50ccs of half strength latex had marked effects.

An exception to the series was found in the case of Al Cl_3, which was found to have comparatively feeble coagulating properties, this is doubtless due to the formation of undissociated Al $(OH)_3$.

(*b*) Acid latex (p- 1.5-2.2) 50 ccs of dilute (18-20% D.R.C.) latex were used in each experiment, with addition of 3cc N.H Cl. Wherever possible, to avoid undue dilution 2 M. solutions of salts were employed and the results calculated back. In Table VIII results are calculated as number of ccs of M. solutions necessary for coagulation within 10 minutes.

TABLE VIII.

Salt used.	Minimum No. of ccs of M. solution required to coagulate 100cc of acid latex.		
	ccs.	Kation equivalent.	Anion equivalent.
NaCl (2M)	10-12	10-12 ccs.	10-12 ccs.
Na_2SO_4 (2M)	3-4	6-8	3-4
K Cl (M)	10-12	10-12	10-12
K_2SO_4 ($\frac{M}{2}$)	3-4	6-8	3-4
Mg Cl. (2M)	4-5	4-5	8-10
Mg SO_4 (2M)	4-5	4-5	4-5
Ca Cl_2. (M)	5-6	5-6	10-12
Ba Cl_2 ($\frac{M}{2}$)	5-6	5-6	10-12
Al Cl_3 (M)	4-5	4-5	12-15
Al K SO_4 ($\frac{M}{2}$)	6-7	6-7 (Al)	6-7
HCl (2M)	20	20	20
H_2SO_4 (2M)	8	16	8

Consideration of these figures shows that the kation is no longer the predominating partner, and that the anion is obviously playing a larger part in precipitation. The kation however, is not absolutely without effect as the highest anion concentration is required for the salts with a cation of the highest valency viz. Al.

Thus along three different lines, viz. viscosity, electric charge and salt effect, and stability we are led to the conclusion that the region of first coagulation is an iso-electric region, while second coagulation is an effect of the anion of the acid employed, which can more readily be brought about by salts.

It is to be noted that instead of a sharply defined iso-electric *point*, there is broad *region* reaching from pH 5.2 to pH 2.2 This blurring is undoubtedly due to the fact mentioned above—that latex is a mixture of many components, including the divalent kations Ca and Mg and the trivalent anion PO_4. Better results could doubtless be obtained after dialysis, but facilities have not, up to the present, being available for dialysis at low temperatures, and preservatives are out of the question.

V. Henri's results on dialysed latex are, as Whitby points out inadmissible, because the specific gravity given by him suggests that the latex used was not that of Hevea.

It is hoped to conduct work on dialysed latex in the future, for the present it suffices to state that latex does undoubtedly show the existence of an isoelectric region.

Surface tension and surface films.

For the same reason that the Ostwald viscosimeter is useless, the capillary tube method of determining surface tension is tedious and unreliable; the drop method was used—in this surface tension is inversely proportional to the number of drops formed. For convenience, the latex used was diluted with an equal volume of water. Table IX gives typical results.

TABLE IX.

Temperature—that of the air = 27°C.

Liquid.	Drop number.
Water ...	31, 31.5, 31
Latex pH 5.9 ...	40, 39
Latex in gap ...	38, 39
Serum of above after coagulation with acetic acid ...	34, 34

Many similar results were obtained, showing a considerable rise in surface tension on coagulation, thus here also latex obeys the conditions set forth.

There can be no doubt of the part played by the protein in emulsification since one or more of these bodies or their closely allied

products,* are precipitated at a pH value very close to that of the first coagulation point. As De Vries points out the action of the protein in forming a protective layer—called for by the theory of surface energy also explains why the properties of latex are those of an emulsoid or hyalophilic mixture, and not those of an unstable sol which might be expected of a highly insoluble terpene.

In the same way the protein layer explains the comparatively high pH value at which the isoelectric region starts, instead of the low value (about 2.2) of most resin and gum sols.

Whitby† has recently pointed out that attention should be given to the 'resins' of latex as possible emulsifiers.

It was found that either the "acetone soluble" or "resin-acids," obtained from latex could be induced to form a "sol" with water by solution in alcohol and then pouring into a large excess of water. This solution was not precipitated at pH 4.5 and remained stable till the value of 2.2—2.4 was reached, when separation occurred.

Another series of experiments dealt with sodium 'resinate.'

The 'acetone soluble' or resin acids were dissolved in alkaline alcohol, the addition of water now gave an opalescent solution, which on the cautious addition of acid, started to precipitate at neutrality and was completely precipitated at pH 6.0. This result indicates that resinates could not exist, as such, in normal latex, and it remains to consider the possible emulsifying action of resin as sol.

Here again we meet with the protein complication, the resin particles of the sol, whether microscopic or ultra microscopic will also be coated by protein, and will therefore have identical properties with the caoutchouc globules, and could therefore not assist in emulsification.‡

There remains the possibility pointed out by Whitby that in *alkaline* latex, the resin may be an additional emulsifier. Confirmatory evidence has been obtained by surface tension determinations.

TABLE X.

Liquid.	Drop number.
Water ...	31, 31.5
Fresh half-strength latex ...	37—40 (range of many samples).
Fresh latex + 2% NH_3	37—38
Latex preserved with 2% NH_3—after 2 months, diluted with an equal volume of 2% Am. Hyreate ...	49—50

* *Vide* accompanying paper on "Some constituents of rubber latex."

† The writer has not had the advantage of reading Whitby's full paper on this subject, and the view here adopted may have been put forward in it.

‡ The question of the independent existence of 'resin' in latex is purposely omitted from this discussion—if it is dissolved in the terpene the question of emulsifying action does not arise. In this connection v. Whitby. Plantation Rubber.

This indicates a gradual change which appears most likely to be the slow conversion of resin acids into resinates.

Theory of Coagulation.

It is suggested that all the phenomona of coagulation of normal latex can be accounted for on the theory that the caoutchouc globules in latex have a protective layer of protein, which has an isoelectric region starting at the first coagulation point of normal latex (pH 4.9—5.4).

'Natural' coagulation is held to be due to acidity developed by bacterial action.

Difficulties.

The apparent difficulties to be encountered by this theory are:—

1. Barrowcliff's experiment.

2. Whitby's observation that tannic acids and other protein *precipitants* (as opposed to coagulating agents) produce at first a slimy coagulum.

3, The fact that the latex of very poor yielders or of trees freshly opened frequently coagulates on the tapping cut, without signs of bacterial action or increased acidity.

To deal with these *seriatim*:—

It has been shown above that the first difficulty is more apparent than real, heating produces 'creaming' and flocculation does occur on increasing the acidity of Barrowcliff latex.

It is admitted by Whitby that once partial separation has occurred the next step—to coagulation—is one of difficulty; the larger aggregates may be in equilibrium with the changed conditions of the dispersion medium, and the number of collisions occurring in a given volume and given time is enormously reduced. In fact a large alteration in the system must be made for the small aggregates to coalesce and coagulation proper to follow—such alterations may be brought about by the addition of comparatively large quantities of salts or acids.

There is a possibility in the case of both (1) and (2), viz—that owing either to the previous action of heat, or the action of tannic acid, the protective protein film is *precipitated* as a definite skin (akin to the membrane of Weber) before the globules have time to coalesce in large numbers. Ordinary coagulation of protein is a slower process which proceeds *pari passu* with coalescence of the globules and does not interfere with the latter. The protective action of alkali in Barrowcliff's experiment supports this view, and may be supposed to render the protein less explosively coagulable.

The third objection may also be met, in both poor yielders and virgin trees the proportion of sap from wounded cells is much higher

than in ordinary latex. This sap contains considerable quantities of Ca and Mg salts and also quantities of acid tannins, and it is more than likely that the combination may cause rapid coagulation. Further investigation on this point will be carried out. It is realised with Whitby that experience of work on latex and the remarkable variations met with does not encourage the making of a universal theory to meet every possible case. The theory set out, does it is believed, meet the conditions met with in ordinary plantation practice.

Deductions from Theory.

It is possible to deduce certain facts of interest from the theory advanced and from facts which have long been known and to enquire how far these deductions are fulfilled in practice.

Coagulants.

(*a*) The acids which by reason of their 'weakness' do not raise acidity to pH 5—4 will not cause coagulation, and may retard it if added in sufficient quantity.

(*b*) Any acid which raises the acidity of latex to a pH value of about 5.0—5.4 will cause coagulation.

(*c*) Only acids which raise the acidity beyond 2.5 will, in suitable quantity suppress coagulation.

(*d*) Only acids which raise the acidity to a higher value than pH 1.5 (?) or which largely increase the anion concentration will again cause coagulation.

All these deductions are capable of verification.

Boric and hydrocyanic acids retard coagulation, acetic acid never reaches the gap, oxalic acid gets into the gap, but not out of it, while the strong mineral acids satisfy condition (*d*).

It is precisely because acetic acid is moderately weak that it is so well suited to plantation practice, where a 'fool-proof' coagulant is needed. Quantities up to one hundred times the correct amount (2% of 5% giving with half-strength latex pH = 5.1 approximately) give a pH value insufficient to cause damage. Slight excess of the cheaper mineral acids causes a large increase of hydrogen ion concentration, and serious disturbances in the subsequent rate of vulcanization.

If the addition of acid could be carried out under laboratory conditions any acid fulfilling condition (*b*) could be used. This was proved by *titrating* latex with several acids, using methyl red as an indicator and the colour given by acetic acid in standard amount as standard (pH = 5.4).

Quantities used were. Latex 2000ccs D.R.C. = 18%.

Acetic acid	40cc N. solution—approx, 20cc 5% to 1000.	
HCl	10 „ N	„
H_2SO_4	11 „ N	„
Oxalic	17 „ M	„
Nitric	11 „ M	„
Tartaric	12 „ M	„
CCl_3COOH	10 „ N	„
H_3PO_4	13 „ (Molar)	

On the following day some of the coagula were softer than others, but on creping drying and vulcanization all gave exactly similar rates of cure and stress-strain curves.

Preservatives.

It is to be deduced that anti-coagulants may act :—

(*a*) By lowering of $H^{\cdot}$ concentration—alkalis.

(*b*) By suppression of rise of $H^{\cdot}$ concentration—buffers and alkalis.

(*c*) By removal of salts which might assist to bring about coagulation—oxalates.

(*d*) By suppression of bacterial action—formalin.

(*e*) By denaturing of protein, rendering it less easily coagulable—formalin.

(*f*) By lowering of surface tension either :—

(i) directly, as saponin.

(ii) indirectly, as alkalis forming resinates.

Also of course there may be a combination of several factors.

It is probable that the most satisfactory preservative will ultimately be found to be a mixture of say—an alkali and antiseptic, and a buffer salt.

A few preliminary laboratory experiments with an alkaline borate mixture gave not unpromising results, but the matter is rather one for a factory trial that for laboratory work.

Any buffer mixture which will keep $H^{\cdot}$ concentration below a given value (about pH 5) will delay coagulation, but once bacterial action starts, sooner or later coagulation will occur.

An example of buffering may be seen below.

TABLE XI.

Citrate-phosphate Buffer.

10cc buffer solution and 10cc strong latex.

pH of buffer solution	...	State of mixture after 24 hours.
1.9	...	Coagulation, no gas.
2.9	...	Coagulation, little gas, few bacteria.
3.5-4.7	...	Coagulation, abundant gas, abundant bacteria.
5.0	...	Some gas, imperfect coagulation.
5.4-5.9	...	Small plug of coagulum, remainder fluid abundant bacteria.
6.3	...	Quite fluid, slight smell of putrefaction.

The absence of the gap is undoubtedly due to the high concentration of salts present.

Brown Bast.

It was stated in the introduction that a study of coagulation might throw light on the most puzzling of all rubber problems viz., brown bast.

It is attractive to speculate on possible causes for the coagulation which occurs within diseased bark—there may be

(*a*) Development of acidity.

(*b*) Raising of surface tension, due to reduction of the protein on overtapping.

(*c*) Raising of the Ca salt concentration in the surrounding bark, with eventual diffusion of excess of Ca into the tubes and coagulation.

This last is particularly attractive in view of the (normally) small amount of Ca removed by latex in comparison with Mg and K, and this might lead to accumulation as suggested.

It is proposed to initiate work on these lines.

Summary.

1. The temperature and volume changes on coagulation are investigated, and reasons given for believing that no change of state occurs on coagulation.

2. Previous theories of coagulation are examined and evidence brought forward that the observations made can be differently interpreted.

3. The conditions necessary for equilibrium and for separation of systems such as latex are considered.

4. Latex is shown to satisfy these conditions.

5. The theory is put forward that coagulation is normally due to increase of hydrogen ion concentration, which may be brought about by bacterial action, and that the phenomena met with can be explained by the assumption of an iso-electric region for the protective protein.

6. The resins are shown to be unlikely to function as emulsifiers in normal latex.

7. Certain difficulties are considered, and deductions of practical interest made.

Literature.

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(2)	Whitby ...	Plantation rubber, 1920 Chapter II pp. 31-75.
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STUDIES ON HEVEA LATEX.

II. SOME CONSTITUENTS OF LATEX.

By W. N. C. Belgrave & R. O. Bishop.

THE work about to be described was the result of an attempt to effect a rough separation of some of the substances in latex with a view to the testing of their effects on the rate of vulcanization of rubber and also as a preliminary survey of possible lines along which more detailed work in the future should proceed. Naturally, final results have not been attained, but it is desired to record the work carried out. The effects of the bodies isolated on vulcanization have been dealt with elsewhere.*

Serum.

When latex is coagulated by the addition of acetic acid a soft open coagulum is formed which subsequently shrinks and toughens. In the first stage the solid holds almost all the liquid and apparently all the gas which exists in the original latex. As a result of the shrinking and toughening of the coagulum the liquid is largely released and can be collected as a clear, practically colourless fluid which is optically active and exhibits a varying laevo-rotation.

On neutralisation with alkali a very distinct change occurs, the neutral point (7.7 pH) being characterised by the development of a deep violet colour which is sufficiently marked to be of use as an indication of neutrality. At the same time a white flocculent precipitate is thrown down. The colour has so far precluded any observations of change of optical rotation in the liquid. The solid can be purified by filtering, washing, solution in dilute acid and reprecipitation. It is found to be a nitrogenous body containing no mineral matter. The nitrogen as estimated by Kjeldahl's method is 12.0%. A diluted acid solution of this substance is not optically active and a precipitate is obtained on heating to 60°C which contains approximately 12% N_2 by Kjeldahl and is not soluble in acid.

A similar precipitate is obtained on heating the original serum to 60°C. On drying it also gives a hard horn-like material which contains no ash and approximately 12% N_2 (Kjeldahl).

A precipitate is obtained on saturation of the serum with Am_2SO_4, but not with Na Cl or Mg SO_4 or on half saturation with Am_2SO_4. The Am_2SO_4 mixture may be taken into a fresh water solution and then by dialysis the sulphate can be separated away. In our work a check on the elimination of the ammonium salt was reckoned to be the complete absence of any precipitate when the dialysate was acidified and tested with Ba Cl_2 solution. The dialysed solution upon

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* M.A.J. XI, No. 11, p. 310.

evaporation on a water bath gives a white solid which on drying is tough, horny and greyish in colour. The nitrogen estimated by Kjeldahl is approximately 12% and there is no ash. The substance gives the typical colour reactions of proteins, and identification with such bodies may, following previous workers, safely be made.

Our work indicates that the serum from a 40% D.R.C.* latex contains approximately 0.15% of this protein, the amount being variable.

An investigation on further chemical characteristics of this body, which for future reference is described as the "heat coagulable protein" to decide whether it as a simple or compound protein (the latter being indicated by the low nitrogen content and the nature of its decomposition products has not yet been attempted).

After the heat-coagulable protein has been removed it is found that prolonged heating at an elevated temperature does not have any further effect on the optical rotation of the liquor. There is no precipitation and no material change in colour, either in acid or neutral solution. We are not convinced however, that all the soluble constituents of the serum liquor remain free from change.

If the liquor (either acid or neutral) is poured into 96% alcohol a white flocculent precipitate is obtained. This on washing and drying is found to be approximately 0.05% of the original latex. The same precipitate is formed from acid or neutral serum which has been subjected to decomposition under aerobic and anaerobic conditions.

The precipitation appears to be due to a coagulation inasmuch as the body cannot be redissolved in the original liquor. It is almost insoluble in water and can be washed free from salts and sugars. Drying in the air causes rapid darkening, drying in vacuo produces a tough horny substance greyish in colour which is found to contain N, Mg, K, P_2O_5. Nitrogen estimated by Kjeldahl is approximately 8.0%. Total mineral matter by incineration is approximately 13.0.

On heating alone in the air the substance decomposes, evolves ammonia and then gases with a strong pynol reaction, and fairly dense brown fumes.

It has not been possible directly to establish the optical activity of this alcohol coagulable body but it is found that after its removal the serum liquid residue exhibits a large reduction in optical rotation although of the same direction as originally determined.

Attempts were made to remove some of the mineral matter of this body by dialysis; for this purpose the original serum liquor remaining after the heat coagulable protein had been separated was subjected to dialysis into running water for several days, after which the alcohol coagulation carried out as usual. The body obtained showed the same percentage of nitrogen (by Kjeldahl) and ash as

* D.R.C. Dry rubber content. Estimated by coagulating with acetic acid.

before, suggesting that the components were combined, or in very close association.

Rubber.

In order to obtain information on the protein associated with coagulated rubber, attempts were made to wash it out of recently coagulated rubber by using water, dilute acid, alkaline and neutral salt solutions.

All attempts at removing appreciable quantities of protein-like bodies from rubber failed, even when the rubber was prepared in exceptionally thin films by spreading latex on filter paper, or by mixing with a large excess of sand and subsequent grinding; similarly it proved impossible to remove suspended, or adsorbed matter from benzene solutions by any mechanical process.

Another method tried was that of mixing dilute latex with ether, and shaking vigorously—when a thick translucent liquid and a clear aqueous solution is obtained—still no effective separation was obtained.

Lastly, the method of Spence and Kratz* was adopted—viz. depolymerisation of an acid benzol solution of rubber by sunlight. A 10% solution of rubber in benzol with .5% trichloracetic acid was employed. After a few days exposure, the previously highly viscous solution became more limpid and eventually (after several days) separated into a perfectly clear golden-yellow solution and a flaky brown sediment. After decantation, this sediment was exposed with fresh acid benzol, then given several washings with benzol, filtered and dried to a brown, somewhat horny mass, which was treated as in Table I.

* v. Whitby Plantation Rubber p. 117.

TABLE I.

The mass was ground, digested with cold water, then filtered.

Filtrate.	Residue. Extracted overnight with cold 1% Na OH, then filtered.			
	Filtrate.		Residue.	
	Yellow solution giving a copious precipitate with dilute HCl. This was filtered.		Extracted for 1 hour with 1% aqueous Na OH at 60°C then filtered.	
	Filtrate.	Precipitate.	Filtrate.	Residue.
Strongly acid. No precipitate on neutralisation or on making alkaline. On evaporation to dryness a pale yellow residue was left, which heated with soda-lime gave copious alkaline vapours and a strong pyrrol reaction. On ashing, there was a copious residue, which reacted for K, Mg, & P_2O_5.	Evaporated to dryness gave a small residue of sodium chloride.	Washed with fainty acid water. Dissolved in dilute alkali reprecipitated with acid and again washed, on evaporation brown resinous flakes were obtained, which on heating with soda-lime gave copious alkaline vapours, and a brilliant pyrrol reaction. On ashing only a negligible residue is left. The substance is entirely soluble in cold alcohol (96%) to a brown solution with marked fluoresence.	Yellow brown colour and apparently identical with the filtrate from the cold extraction.	Very small in amount—burns with a strong smell of rubber—leaving no appreciable ash—and giving traces only of nitrogenous bodies when heated with soda-lime.

The very surprising solubility in alcohol of the supposed protein precludes the idea that the substance obtained by us on depolymerisation was unchanged protein—it leaves open the question of derivation. Does the separation of a nitrogenous material with much ash soluble in water irrespective of reaction from the ashless body soluble only in alkaline solution represent an original separation, or are they both breakdown products of a parent body—the splitting being due to prolonged insolation? The problem cannot be answered at this stage.

It may be of interest to note that the benzol rubber solution on evaporation left a sticky brown residue which had an N content (by Kjeldahl) of only .07%. After standing in bright diffuse light in an open beaker for 2—3 months this residue became tough and moderately elastic and was clear brown in colour. It vulcanized with marked rapidity and the character of the curve obtained on the Schopper machine was normal.

Alcoholic mother liquors.

Attention was next turned to the alcoholic liquors—as described elsewhere* the alcohol was 'acid' pH *c.* 3 and 'neutral' pH *c.* 7.4 and 'alkaline' = *c.* 9.0.

The solutions were filtered to remove traces of suspended rubber and were faintly yellow in colour. They were then distilled to remove most of the alcohol, becoming turbid in the process, and at the same time turning pale green in the case of 'acid' or 'neutral' solutions, pale red-brown when 'alkaline.' They were further concentrated on a water-bath, with a progressive separation of waxy-looking matter which in acid or neutral solution, lumps together and may be readily removed by filtration. In alkaline solution—lumping does not occur, and filtration is almost impossible, except after addition of acid,—it is hoped shortly to effect separation in alkaline solution by the use of a high speed centrifuge.

Waxy solids insoluble in water.

The waxy solid obtained by such treatment is now only partially soluble in alcohol, suggesting that heating to 90°C has caused decomposition, this residue was further treated as follows:—

Solid extracted with acetone.

Solution.

Yellow brown, giving on evaporation a dark brown waxy mass—called herewith "acetone-soluble."

Residue. Extracted with hot chloroform.

Solution.	*Residue.*
Bright yellow giving yellow waxy mass on evaporation called "lecithin-body."	Light brown friable solid, insoluble in water or alcohol called "insoluble."

* M.A.J. XI, p. 11.

Acetone soluble.

Is entirely soluble in alcohol, and if this solution is poured into excess of water, a very slightly opalescent liquid of lowered surface tension is obtained. On addition of acid or of salts of alkaline earth metals a white precipitate is thrown down. The original material is soluble in hot aqueous alkali, on addition of acid a yellow white non-N precipitate is obtained; the solution on heating with strong alkalies evolves NH_3. On evaporation and heating with soda lime no pyrrol reaction is obtained. A negligible residue is left on ashing.

After saponification with alcoholic potash in the usual way the unsaponifiable residue gave strongly marked cholesterol reactions and is undoubtly composed of the plant alcohols described by Whitby.

The residue is partly composed of acid bodies (resin acids) and partly of sugar and nitrogenous bodies.

Lecithin-body.

From the method of preparation, is obviously insoluble in acetone, is easily soluble in alcohol, and also forms an opalescent solution on pouring the latter into water—from which it is precipitated by acid.

On heating with caustic soda solution or soda-lime copious alkaline vapour with a strong fishy smell are evolved—no pyrrol reaction is given. On ashing, a residue is left which gives a strong reaction for phosphate.

In alcoholic solution a crystalline precipitate is obtained with cadmium chloride. It seems safe to assume that this body is akin to "lecithin."

Insoluble.

The fact that this body is produced by heating was confirmed by conducting the evaporation of the original alcoholic liquor in vacuo at a temperature not exceeding 45°C. Separation of the waxy bodies took place but these was entirely soluble in alcohol, and practically ashless.

The only one of the usual colour tests for N bolies given by the "insoluble" was the zanthoproteic; murexide, Millon and biuret were negative.

On heating alone or with soda lime gives some alkaline vapours and a brilliant pyrrol reaction. On ashing the residue consists almost entirely of magnesium phosphate. The amounts of the three bodies obtained by dropping 1000 ccs latex (40% dry rubber) into 3000 ccs warm neutral alcohol, with their N and ash content are given below.

Acetone soluble.	Lecithin-body.	Insoluble.
Grams 3.6	2.0	0.9
N % (K) .9%	1.2%	10%
Ash trace	6.5%	3%

It will be noted that the "insoluble" from alcohol contained more N and less ash than that from serum. More detailed analytical work will to carried out in the future. It is suggested that both "insolubles" represent some form of humin break-down product, combined with, or very closely adsorbing mineral constituents.

Water-soluble.

On evaporation and ashing, the water soluble residue after removal of the waxy bodies gave Mg, K, traces of Ca, and P_2O_5. On heating alone, alkaline vapours and the pyrrol reaction were obtained and these were increased by heating with soda lime.

The water soluble portion on evaporation gave non-N crystals in a brown sticky matrix and all attempts at an effective separation of nitrogen bodies from the matrix failed. Lead acetate, phosphotungstate, mercuric chloride and similar reagents, give precipitates which on heating with soda lime evolved alkaline vapours but gave no pyrrol reaction.

These precipitates are probably from decomposed protein products but the residue after precipitation with these and other precipitants invariable gives a strong pyrrol reaction and the reacting body is obviously dissolved in the brown uncrystallible mother liquer. On account of the possible theoretical importance of pyrrol-ring bodies in the synthesis of caoutchouc prolonged but fruitless attempt were made to separate such bodies by extraction with the usual organic and other solvents from acid and alkaline residues. This elusiveness suggests the possibility that the pyrrol test obtained is due only to a secondary reaction between ammonium salts or amines and laevulinic aldehyde present in the residue; the latter body being a well known decomposition product of oxidised caoutchouc—apart from the possibility of formation from carbohydrate.

The alcoholic solution was was accordingly tested for laevulin -aldehyde with negative results.

To sum up, in latex there appears to be N in the form of protein bodies, lower diffusible products thereof and lecithin-like bodies. The intensity of the pyrrol reaction almost always obtained, the solubility in alcohol of products giving it, and the low percentage of N invariably obtained in separated products, suggest that the proteins in latex are not of the usual type. Further experiments are in hand with a view to better methods of protein extraction.

NITROGEN DETERMINATIONS ON LATEX AND RUBBER.

The uncertainty of the form in which N occurs in latex suggests that Kjeldahl's method should not be relied on as a measure of the total N. in latex or rubber and a series of determinations by Dumas' method were accordingly carried out.

Preliminary determinations with a commercial sample of acetanilide gave good agreement between the two methods.

	Dumas nitrogen %	Kjeldahl nitrogen %
Acetanilide	12.0	12.05
Latex sample A	0.76	.26
„ B	0.68	.25 & .25
„ C	0.72	.36
„ D	0.80	—

The estimation is not without difficulty, and owing to the large carbon and small nitrogen content of latex it is inadvisable to mix the latter with fine CuO as reduction once started is apt to be violent, and in one case led to a minature explosion.

The agreement of the Dumas results above is so good, considering the varying nature of the product that it was considered to be proved that far more N is present in latex than is shown by Kjeldahl. Estimations were then carried out on fresh crepe, and gave results varying from .76 to 1.2% of N against the Kjeldahl figure of .25%. Again the agreement of the Dumas' results seemed within expectation. As a final test, eudiometric examination of the evolved gas were carried out, and in every case, with rubber, an explosion followed on sparking with O i.e. reduction had been incomplete. So far no modifications of the apparatus using CuO has given non-explosive gas. It is hoped to get better results with some of the newer Pt contact methods, until this has been done no reliance can be placed on any of the Dumas' results given above even though in all comes the contraction following explosion was very small. Unfortunately, no test was made with the gas from latex, so for the present these results also must be discarded.

Inosite-bodies.

Several previous workers have recorded the presence of l-me-inosite in latex. This was confirmed by the writers, who have however, so far, failed to obtain the characteristic colour reaction of inosite with the methyl compound. This reaction however, is known to be very easily upset by trace of impurities ; or possibly, by methylation.

It is of interest to note that the observation recorded above that there is a loss of rotation of serum after removal of an 'insoluble' body—invalidates Gorter's* method of estimating l-me inosite in serum by determination of rotation.

SUMMARY.

An account is given of the separation of :—

(1) Serum protein on neutralisation.
(2) an alcohol-insoluble nitrogenous body from serum.
(3) different constituents of the 'depolymerised residue.
(4) waxy bodies—phytosterols, resin acids and a lecithin—like body from alcoholic liquors.

* v. Whitby Plantation Rubber p. 63.

Reasons are given for thinking that some of the supposed proteins separated are either break-down products or are different in composition from proteins normally met with.

Unsuccessful attempts at Dumas estimation are described.

Received for publication 20th November, 1923.

NOTE.

Passiflora.

The Department of Agriculture, S.S. and F.M.S. is now in a position to supply a limited number of plants of *Passiflora edulis*, *Passiflora quadrangularis* and *Passiflora laurifolia*.

Application should be made to the Agriculturist, Department of Agriculture, S.S. and F.M.S.

Centrosema.

The Department of Agriculture, S.S. and F.M.S. has reduced the price of *Centrosema Plumieri* seed from 14 cents per lb. to 8 cents per lb.

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The following publications, except those out of print, may be obtained on application to the Office of the Secretary for Agriculture: the Raffles Museum, Singapore; and the Malay States Information Agency, 88, Cannon Street, E.C. London.

A remittance to cover the cost should accompany applications; otherwise the Journals will be sent by post, Cash on Delivery, where that system is in force.

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